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14. ABSTRACT

This research project focuses on prostate cancer, a devastating socioeconomic disease, whose detection is plagued with inadequate sensitivity and specificity. Hypoxia is the hallmark of malignancy because aggressive cancers outgrow their blood supply. We ultimately aim to build an instrument that combines OPtics and UltraSound (OPUS) to quantify hypoxia via optical imaging but with the improved spatial resolution of US imaging. Specifically, the acousto-optic effect will be used to only modulate light (at the ultrasound frequency) which propagates through a small ultrasound focal zone. This DOD Idea Development Award is concerned with the development of a novel acousto-optic detection idea based on quadrature measurements with a gain-modulated image intensified CCD camera. Furthermore, we proposed the novel idea of using microbubble-based contrast agents to significantly increase the light modulation and, moreover, the use of fluorescent microbubbles to provide additional enhancement. During the first year of the research project we have demonstrated the detection of ultrasound-modulated incoherent photons followed by the novel quadrature detection of ultrasound-modulated photons and fluorescence photons with the gain-modulated image intensified CCD camera approach. This research demonstrates the potential to perform acousto-optic molecular imaging of prostate cancer with incoherent and fluorescence photons using endogenous contrast, e.g. hypoxia, and also fluorescent probes.

15. SUBJECT TERMS
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INTRODUCTION

This research project focuses on prostate cancer, a devastating socioeconomic disease, whose detection is plagued with inadequate sensitivity and specificity. Hypoxia is the hallmark of malignancy because aggressive cancers outgrow their blood supply. Optical imaging is emerging as a physiologic tool capable of quantifying hypoxia but only at centimeter spatial resolution which is unacceptable for prostate cancer imaging. We ultimately aim to build an instrument that combines OPtics and UltraSound (OPUS) to quantify hypoxia via optical imaging but with the improved spatial resolution of US imaging. Specifically, the acousto-optic effect will be used to only modulate light (at the ultrasound frequency) which propagates through a small ultrasound focal zone. Optical images generated from only ultrasound-modulated light will thus have the improved spatial resolution of the ultrasound focal zone. The main difficulty is the detection and discrimination of ultrasound-modulated light from the overwhelming presence of non-modulated light not passing through the ultrasound focal zone. This DOD Idea Development Award is concerned with the development of a novel detection idea based on quadrature measurements with a gain-modulated image intensified CCD camera. Furthermore, we proposed the novel idea of using microbubble-based contrast agents to significantly increase the light modulation and, moreover, the use of fluorescent microbubbles to provide additional enhancement.

BODY

The Statement of Work outlined four main tasks for this project. The following describes the research conducted for each task during this reporting period:

Task 1. Prove that modulating optical signal amplification with the US frequency to preferentially amplify the modulated photons results in improved SNR.

For acousto-optic imaging, the size and location of the US-focal zone provides the image spatial resolution and the optical detector is simply used to collect as many modulated photons as possible to improve SNR. For the detection of ultrasound-modulated coherent light, simple use of a collection lens to focus many speckles to a single detector results in phase-cancellation which ultimately eliminates the modulation signal rather than increasing it. Hence, some researchers[1] have used a CCD camera for parallel detection where each pixel acts as an independent detector to detect a larger number of speckles. However, since the frame-rates of CCD cameras are not capable of monitoring the speckle modulation via ultrasound (MHz) in real-time, some researchers[2] examine the speckle contrast of a single CCD frame which is inversely proportional to the magnitude of the acousto-optic effect.

As hypothesized in task 1, we expected the use of an image intensified CCD camera gain-modulated at the ultrasound frequency would lead to a greater change in the speckle contrast correlated with increasing the detector gain. These initial experiments employed a laser diode (5 mW at 650 nm, coherence length of ~5 M) to illuminate a water tank (65 mm wide) to which we added varying amounts of intralipid as an optical scatterer. Light was detected with a gain-modulated image intensifier (Picostar HRI, LaVision, Germany) with a highly sensitive electron-multiplying CCD camera (Andor, CA) to detect the output of the image intensifier and store it on the acquisition computer. Modulation of the image intensifier gain was achieved with a function generator (Stanford Research Systems, DS345) outputting a sine wave at the ultrasound frequency and phase-locked ($\Delta\Phi = 0$) to a second identical function generator input to an RF Amplifier (ENI, 240L) to drive a single-element ultrasound transducer (Panametrics, V303) with a 1 MHz sine wave (50 Vpp) with 20 pulses (20 μs pulse-length) every millisecond (2% duty cycle). The US transducer was submerged in the water tank such that its focal zone (mechanically focused to 1.5 inches) intercepted the line-of-sight between the laser diode and the image intensifier in transmission mode. Since speckles are typically around 10 μm (similar size to 8μM CCD pixel) we used an extension tube (PN-11, Nikon) and 50 mm f/1.4 Lens (Nikon) to achieve 1:1 imaging (i.e. 8 μm spatial resolution) over an 8 x 8 mm(1000 by 1000 pixels) field of view. The experimental configuration is shown in Figure 1.

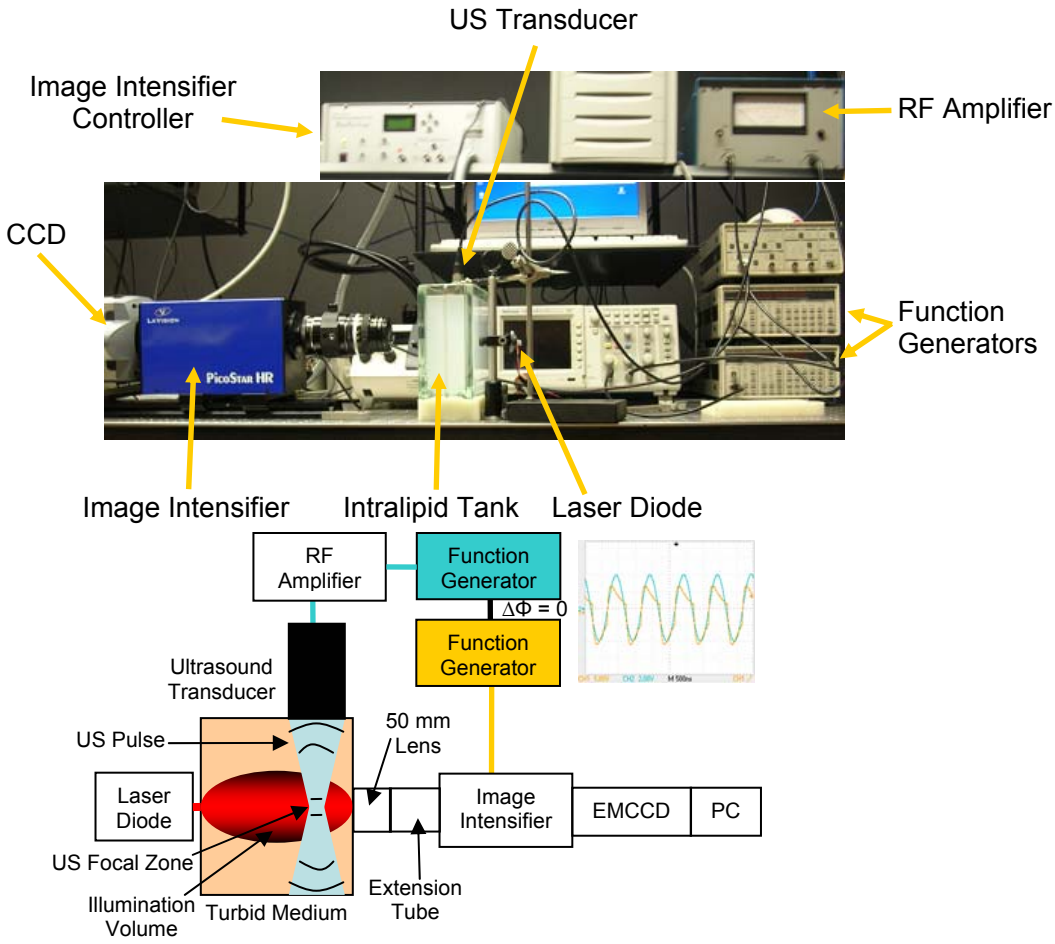
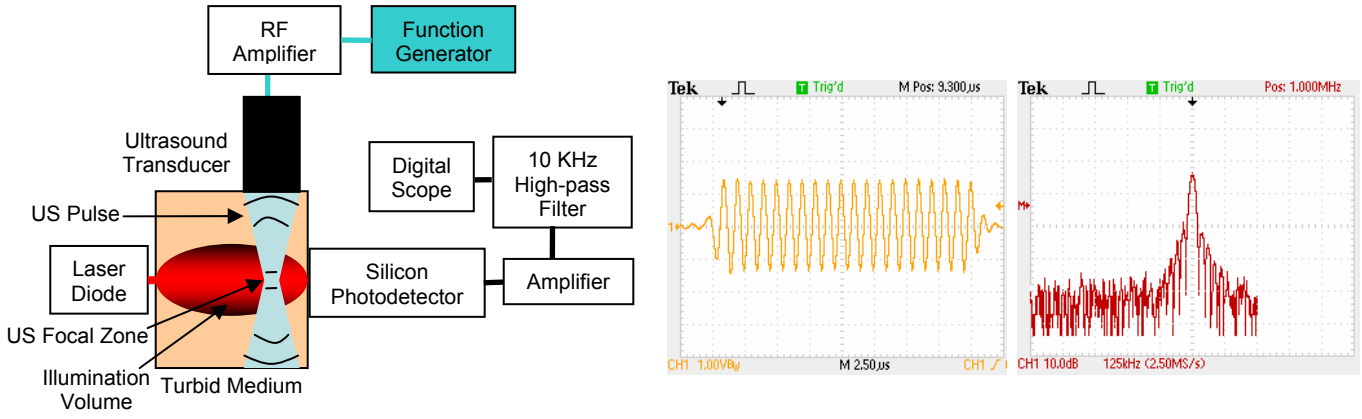


Figure 1 a) Experimental configuration for acousto-optic detection with gain-modulated image intensifier 1b) Schematic representation

A baseline measurement was first performed with no gain modulation, i.e. a steady-state gain, with and without the ultrasound being applied to measure the change in speckle contrast. The image intensifier was set to 300 V, the CCD integration time to 400 ms, and 100 sequential images were acquired. The speckle contrast is defined as the standard deviation of the image intensity (σ) divided by the mean intensity (<I>). Without ultrasound we measured an average speckle contrast of 0.03557 and with ultrasound an average speckle contrast of 0.03548 demonstrating the decrease as expected. However, upon further analysis we found the standard deviation in the speckle contrast across the 100 images was 0.0002 and so this decrease was within the measurement error. We explored a variety of image intensifier voltages and CCD integration times but were unable to detect any statistically meaningful difference in the speckle contrast with and without ultrasound being applied. This result was somewhat surprising, but undaunted we proceeded to try and detect a change in speckle contrast when applying a gain-modulation to the image intensifier. The image intensifier is comprised of a microchannel plate (MCP) held at a high amplification voltage and a photocathode whose response is modulated by the function generator. Unfortunately, even with full modulation of the photocathode we were still unable to detect any difference in the speckle contrast with and without ultrasound being applied. This result was unexpected and somewhat concerning, causing us to recheck that the equipment was indeed operating correctly.

At this stage of the project we had been unable to detect the elusive ultrasound-modulated photons and decided to simplify the set-up to a more basic experimental configuration. The gain-modulated image intensified CCD was replaced with an amplified (20 dB) silicon photodetector (PDA36A, ThorLabs). Although this is essentially only one pixel compared to the CCD camera, it has sufficient bandwidth (MHz range) to follow the signal in real time which the CCD cannot. Unlike the previous experiment which resolved individual speckles, we simply detected all the transmitted light over ~ 1 cm^2 area. The output of the silicon photodetector was further amplified and passed through a high pass-filter(Stanford Research Systems, SR560), >10 KHz, to remove DC and low frequency noise before being recorded on a digital scope (Tektronix, TDS 2012B). Under

this experimental configuration, Figure 2a, we were finally able to detect the ultrasound-modulated optical signal which was recorded on the scope (Fig. 2b). A Fourier transform of this signal (Fig. 2c) clearly shows a peak at 1 MHz (the ultrasound frequency) where the amplitude is proportional to the magnitude of the acousto-optic effect. As a control, the measurement was repeated without the ultrasound being applied for which there was no modulation detected in the optical signal.



(a) *(b)* *(c)*

Figure 2a) Experimental set-up for detection of ultrasound-modulated coherent light with a silicon photodetector
2b) Optical modulation detected and recorded on the digital scope.
2c) Fourier transform demonstrating optical modulation is at the US modulation frequency.

This experimental result is critical and demonstrates that we can detect ultrasound-modulated photons when all the individual speckles are spatially integrated on the detector. As discussed above, the spatial integration of random speckles leads to phase-cancellation which should destroy any modulation. This led us to speculate that the modulation observed here is not due to modulated speckles, caused by modulating the interference pattern of coherent photons, but rather, due to modulation of the local optical attenuation which essentially modulates the intensity of the photons in phase. The optical attenuation can be modulated via changes in the refractive index, optical absorption and scattering, and/or density of scatters by the ultrasound wave. Furthermore, modulation of the optical attenuation should modulate photon intensity regardless of whether the photons are coherent or incoherent.

To date, most acousto-optic researchers[1] have relied upon the use of coherent photons with the necessity for photon coherence from optical source to ultrasound-focal-zone to optical detector imposing a requirement on the laser to have a long coherence length (at least as long as the optical pathlength). For highly scattering media, the optical pathlength can easily reach six times the geometrical distance[3] restricting the choice of available lasers. The ability to intensity-modulate incoherent photons with ultrasound removes this constraint permitting a wider range of optical sources to be considered. Moreover, since the fluorescence process is inherently incoherent, it allows the potential for ultrasound-modulation of fluorescence photons. We therefore modified our experimental set-up, Figure 3a, to demonstrate the detection of ultrasound-modulated incoherent photons. We simply replaced our coherent laser diode (Figure 2a) with an incoherent light source (100 W Halogen Lamp, Schott KL1500). As expected, Figure 3b show the detected and recorded modulation of the optical signal which exhibits modulation at the ultrasound frequency of 1 MHz. Note, the envelope is caused by a lower frequency modulation from the lamp that was not blocked by our frequency filter.

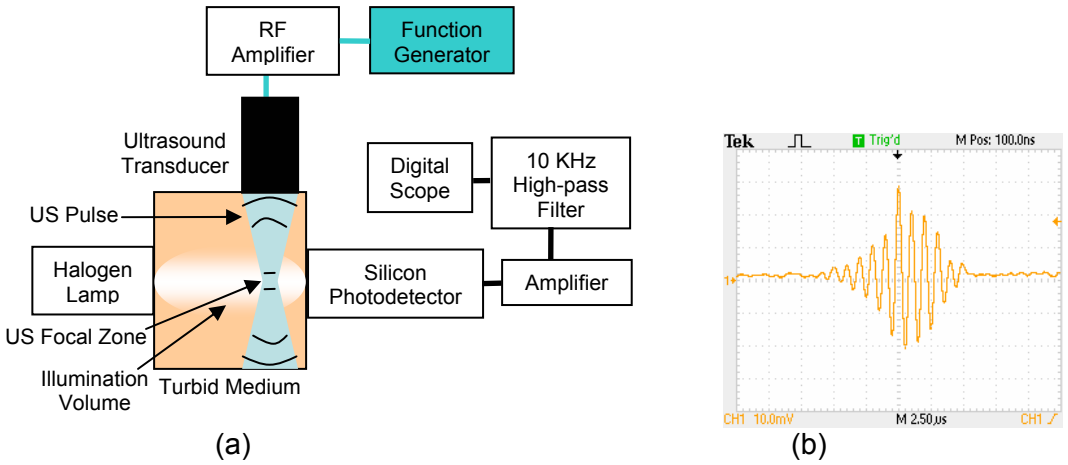


Figure 3a) Experimental set-up for AO measurements of ultrasound-modulated incoherent light. 3b) Optical modulation at 1 MHz detected and recorded on the digital scope.

Although we did not detect the expected change in speckle contrast and could therefore not demonstrate an improvement in SNR, this research led to the novel ultrasound-modulation of incoherent photons. Moreover, the idea that all the photons are modulated in phase by modulation of the optical attenuation explains why we did not observe any change in speckle contrast when applying ultrasound. The decrease in speckle contrast from coherent photons by the addition of ultrasound observed by others[2] relies on the principle that the standard deviation of the intensity decreases while the average intensity remains unaffected as all the random phases of the speckles cancel out. However, a modulation of the optical attenuation by ultrasound causes an in phase intensity modulation which simply scales the standard deviation and average intensity such that the speckle contrast remains unchanged as we observed.

Task 2. **Prove that the use of a Picostar camera system and a quadrature technique results in faster data acquisition without loss of SNR.**

Particularly novel to this project is the proposed quadrature detection of ultrasound-modulated photons with a gain-modulated image intensifier to detect ultrasound-modulated photons[4]. Here, a continuous wave laser source is employed and the gain of the optical detector is modulated at the same frequency as the ultrasound. The CCD image is then acquired at four different phase shifts between the optical detector modulation and ultrasound modulation. The idea is inspired by the way wide-field fluorescence lifetime imaging microscopy (FLIM) is performed[5]. For this application, Fig. 4, a modulated (~ hundreds of MHz) light-emitting diode (LED) source is employed to excite a fluorophore resulting in a modulated fluorescence signal where the fluorophore lifetime induces a phase shift. Hence, the measurement of phase shift at each pixel enables a fluorescence lifetime image to be generated.

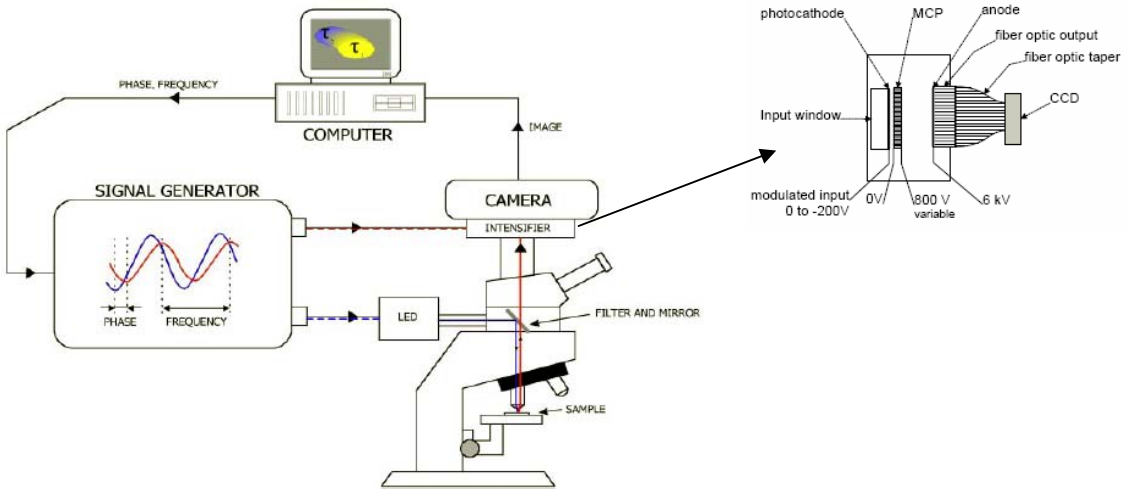
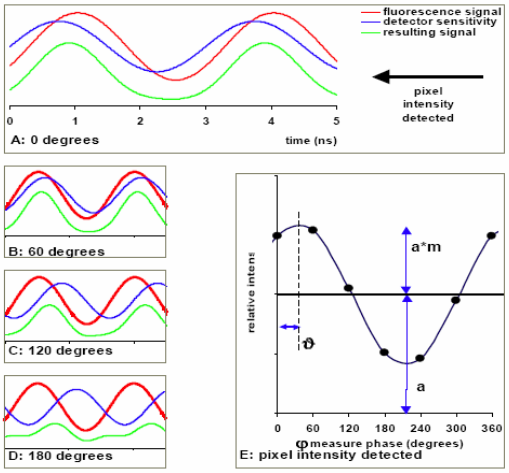


Figure 4. FLIM system setup: Wide-field fluorescence microscope and LIFA attachment for FLIM



The measurement of phase shift is achieved by modulating the photocathode of the image intensifier at the same frequency as the LED. The resultant signal detected on the CCD pixel is thus a product of the phase-shifted fluorescence signal and the optical detector sensitivity. However, a time-averaged signal is recorded since a CCD does not have sufficient frame-rate to follow this signal. Nevertheless, by acquiring this signal at four different phases, Fig 5, one is able to measure the cross correlation of the fluorescence signal and the optical detector sensitivity. The cross-correlation enables the amplitude and phase shift of the fluorescence signal and hence the fluorophore lifetime to be measured.

Figure 5. Cross-Correlation Principle

For acousto-optic imaging, one can simply substitute the fluorescence signal in the FLIM example with the ultrasound-modulated light signal (albeit at 1 MHz not hundreds of MHz). Thus the amplitude, A, of the acousto-optic effect can be calculated directly from the following equation:

$$I = S0 - S180$$

$$Q = S90 - S270$$

$$A = \sqrt{(I^2 + Q^2)}$$

where Sn is the CCD image acquired for a given phase-shift of n degrees.

For ultrasound-modulated incoherent light the photons are modulated in the US focal zone due to a modulation in the optical attenuation. Since photons then take different pathlengths from the US focal zone to the optical detector there are still residual phase differences in the resultant optical signal which could be corrected for with this technique. However, at 1MHz these phase differences were found to be negligible, especially compared to those typically found in standard frequency domain optical imaging using a modulated laser source and conducted at hundreds of MHz [6]. Hence, even without performing phase-alignment, the spatial integration of the signal from this large area detector is still a preferable and more efficient option for capturing the ultrasound-modulated photons than using a small area, single photodetector.

The experimental set-up for the gain-modulated image intensifier approach is shown in Fig. 6:

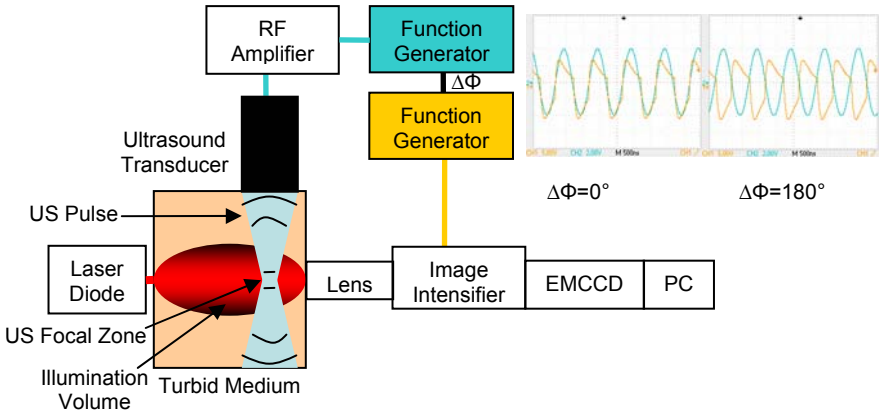


Figure 6. Experimental configuration for acousto-optic detection with gain-modulated image intensifier

The configuration is similar to that described in Figure 1, except that the extension tube has been removed since we now want to collect the optical signal over a much larger optical area (several cm^2) rather than focusing on individual speckles. Based on our previous experiments with the silicon photodiode we used the coherent source, rather than the halogen lamp, since it provided a more stable CW source. The coherence of

the source is not relevant here since we are not observing speckle contrast but rather the modulated photon intensity via ultrasound-modulation of the optical attenuation. Figure 6 shows the driving signals to the ultrasound (blue) and the image-intensifier (yellow) for the two cases of in phase ($\Delta\Phi$=0°) and out of phase ($\Delta\Phi$=180°). For quadrature detection, by definition, four phase measurements are generally required as described above. However, here we intentionally phase-aligned the signals for the special case that the maximum and minimum signals coincided with $\Delta\Phi$=0° and $\Delta\Phi$=180° respectively. As such, the amplitude of the acousto-optic effect is simply the difference between the two measurements. The image intensifier's microchannel plate (MCP) was set to a voltage of 260 V (low) and the CCD integration time was set to 320 ms per frame. Several images were then acquired for $\Delta\Phi$=0° before switching to $\Delta\Phi$=180° and finally with the ultrasound turned-off as a control. The resultant CCD images were then spatially integrated and the intensity values, I, are displayed in Fig 7:

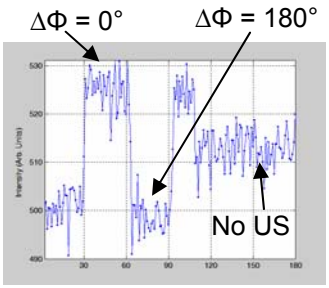


Figure 7: Detection of US-modulated photons with Gain-modulated image intensifier

This result clearly demonstrates that ultrasound-modulation causes a decrease in the local optical attenuation when $\Delta\Phi$=0° resulting in higher intensity, and an increase in the local optical attenuation when $\Delta\Phi$=180° resulting in lower intensity, compared to the local optical attenuation and intensity when no ultrasound is applied. Note, we still switched the detector's phase back and forth when no ultrasound was applied as a further control. Also, the intensity obtained without US being applied is midway between $\Delta\Phi$=0° and $\Delta\Phi$=180° as expected. The modulation in the optical intensity due to ultrasound can best be represented by the induced modulation depth, M, equal to ($I\Delta\Phi0° - I\Delta\Phi180°$)/($I\Delta\Phi0° + I\Delta\Phi180°$) which here is approximately 3%.

This, to our knowledge, is the first detection of ultrasound-modulated photons using a gain-modulated image intensifier phase detection approach.

Ultrasound-Modulated Fluorescence

The detection of ultrasound-modulated fluorescence would promote acousto-optic imaging to acousto-optic molecular imaging with the use of targeted fluorophore-based optical probes. As mentioned above, fluorescence is an incoherent phenomenon and we had previously detected ultrasound-modulation of incoherent photons with the silicon photodetector (Fig. 3). However, this had employed a 100W halolgen lamp which is many orders of magnitude brighter than a typical fluorescence signal, nW. Hence, although we did attempt it, it was not surprising that we were unable to detect a fluorescence signal with the silicon photodetector. Fluorescence detection usually requires a more sensitive optical detector, such as a photomultiplier tube (PMT) or microchannel plate (MCP), due to the low quantum yield of common fluorophores. To our knowledge only two other groups [7, 8] have recently reported on the detection of ultrasound-modulated fluorescence and both used a single detector as opposed to, and without the benefits of, our large area, multi-pixel image intensifier. We have previously used our image intensifier to detect time domain fluorescence signals[9] and now planned to use it to detect ultrasound-modulated fluorescence. We chose a small cylindrical fluorescent pellet, 15 mm diameter by 8 mm height, containing the fluorophore Qdot800 (Invitrogen, CA) at 10 pM concentration. Our manufacture of such pellets is reported elsewhere[10]. The fluorescent pellet was placed on the side of the water tank in the line of sight between the laser diode and optical detector to which we had added a wavelength filter (OmegaFilters) to block detection of the laser diode excitation light and permit detection of fluorescence from the fluorescent pellet (see Fig. 8).

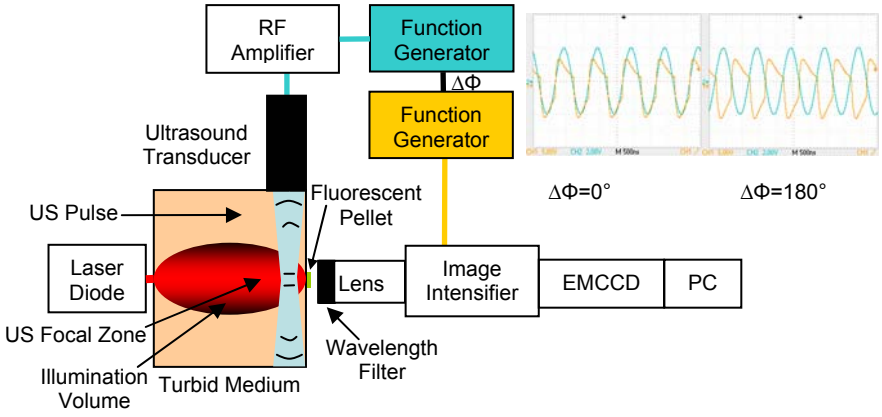


Figure 8: Experimental set-up for detection of ultrasound-modulated fluorescence

Due to the weak fluorescence signal we increased the MCP voltage to 510 V (medium) and acquired several images with an integration time of 320 ms at ΔΦ=0° before switching to ΔΦ=180°. The resultant CCD images were then integrated and the intensity values are displayed in Fig 9:

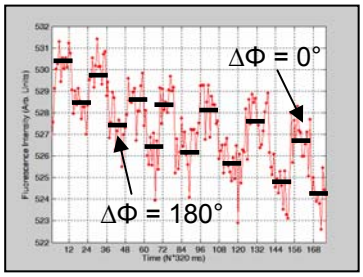


Figure 9: Detection of US-modulated fluorescence with Gain-modulated image intensifier

This result demonstrates the detection of ultrasound-modulated fluorescence with the gain-modulated image intensifier. Note the modulation depth is much weaker, approximately 0.2 %, compared to that measured from excitation photons (see Figure 7) and is expected since we now have a weaker fluorescence signal. Indeed, at these low light levels a systematic downwards drift in the detector's response is also evident. This is not a concern since simple data-processing can remove this baseline effect. As before, without the ultrasound being applied there was no modulation detected. It should be noted that the fluorescent pellet is actually located just after the ultrasound focal zone and closer to the detector to ensure a strong detected optical signal, rather than submerged within the focal zone itself. As such, the fluorescence photons are not being modulated per se, but rather the excitation photons from the laser diode are being modulated just before the pellet which then induces a modulation in the fluorescence signal. Indeed, even if the fluorescent pellet and focal zone were exactly co-localized the dominant modulation of the resultant fluorescence signal would be due to modulation of the excitation photons rather than modulation of the emitted fluorescence photons themselves. One can consider the continuous wave photons from the optical source forming a localized modulated optical source in the ultrasound focal zone which, when in close proximity of a fluorophore, generates a modulated fluorescence signal which would otherwise be a steady-state fluorescence signal. Hence, there is potential to image fluorophore distribution with ultrasound spatial resolution. It should be noted that a slight misalignment in the US focal zone proximity to the fluorescent pellet led to a total loss of the modulated fluorescence signal demonstrating that the method should provide a very high spatial resolution (~mm).

This, to our knowledge, is the first detection of ultrasound-modulated fluorescence photons using a gain-modulated image intensifier phase detection approach.

Task 3. Prove that microbubble-based US contrast agents can increase the photon modulation and result in higher SNR in regions containing microbubbles.

As planned, task 3 will be conducted during the second year of the research project.

Task 4. Prove that fluorophores attached to microbubbles will result in modulation of the photons at the emission frequency.

As planned, task 4 will be conducted during the second year of the research project. As presented in task 2, we have already demonstrated the detection of ultrasound-modulated photons at the fluorescence emission frequency and plan to develop and employ fluorophore-based microbubbles.

KEY RESEARCH ACCOMPLISHMENTS

1. Detection of ultrasound-modulated incoherent photons.

2. Novel quadrature detection of ultrasound-modulated photons with a gain-modulated image-intensified CCD.

3. Novel quadrature detection of ultrasound-modulated fluorescence photons with a gain-modulated image-intensified CCD.

REPORTABLE OUTCOMES

Publications:

Hall, D.J. and U. Sunar, *A novel acousto-optic molecular imaging system for fluorescence-based probes.* Joint (SMI&AMI) Molecular Imaging Conference, 2007.
(Triple blind peer-review scored as one of the Top Ten conference abstracts out of ~1000 presentations).

Hall, D. J. and U. Sunar, *Quadrature detection of ultrasound-modulated photons with a gain-modulated image-intensified CCD camera* (In Preparation 2008)

Grants Applied for:

NIH: U54 RFA-CA-08-002 NTR: Optical Imaging in Multimodal Platforms.
“High Resolution Optical-Ultrasound (OPUS 1) Molecular Imaging in Breast Cancer
R. Mattrey and D. Hall (Co-PIs). Employed research supported by this award as Preliminary Data.

CONCLUSION

The first year of this research project has involved the detection of ultrasound-modulated incoherent photons, based on the principle of modulating the optical attenuation, followed by the novel quadrature detection of ultrasound-modulated photons and fluorescence photons with a gain-modulated image intensified CCD. This research demonstrates the potential to perform acousto-optic imaging with incoherent and fluorescence photons providing novel opportunities for in vivo acousto-optic molecular imaging based on endogenous contrast and fluorescent probes. This cutting-edge research, presented at the Joint Molecular Imaging conference[4] and peer-review rated as one of the top ten abstracts out of ~1000 presentations, is currently under preparation for peer-reviewed publication.

In the second year of this research project we intend to investigate the novel addition of microbubbles (task 3) to amplify the ultrasound-modulation and increase sensitivity. The further extension to fluorophore-based microbubbles (task 4) should provide increased specificity.

Ultimately, acousto-optic imaging could be used to diagnose prostate cancer in vivo based on optical contrast of endogenous hypoxia and/or cancer-targeted fluorescent microbubbles at ultrasound spatial resolution. We have recently submitted an NIH U54 grant proposal for the 5-year development of a clinical prototype.

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